Figure 1. Melting Curves for Genotyping
the Most Stable Mismatch
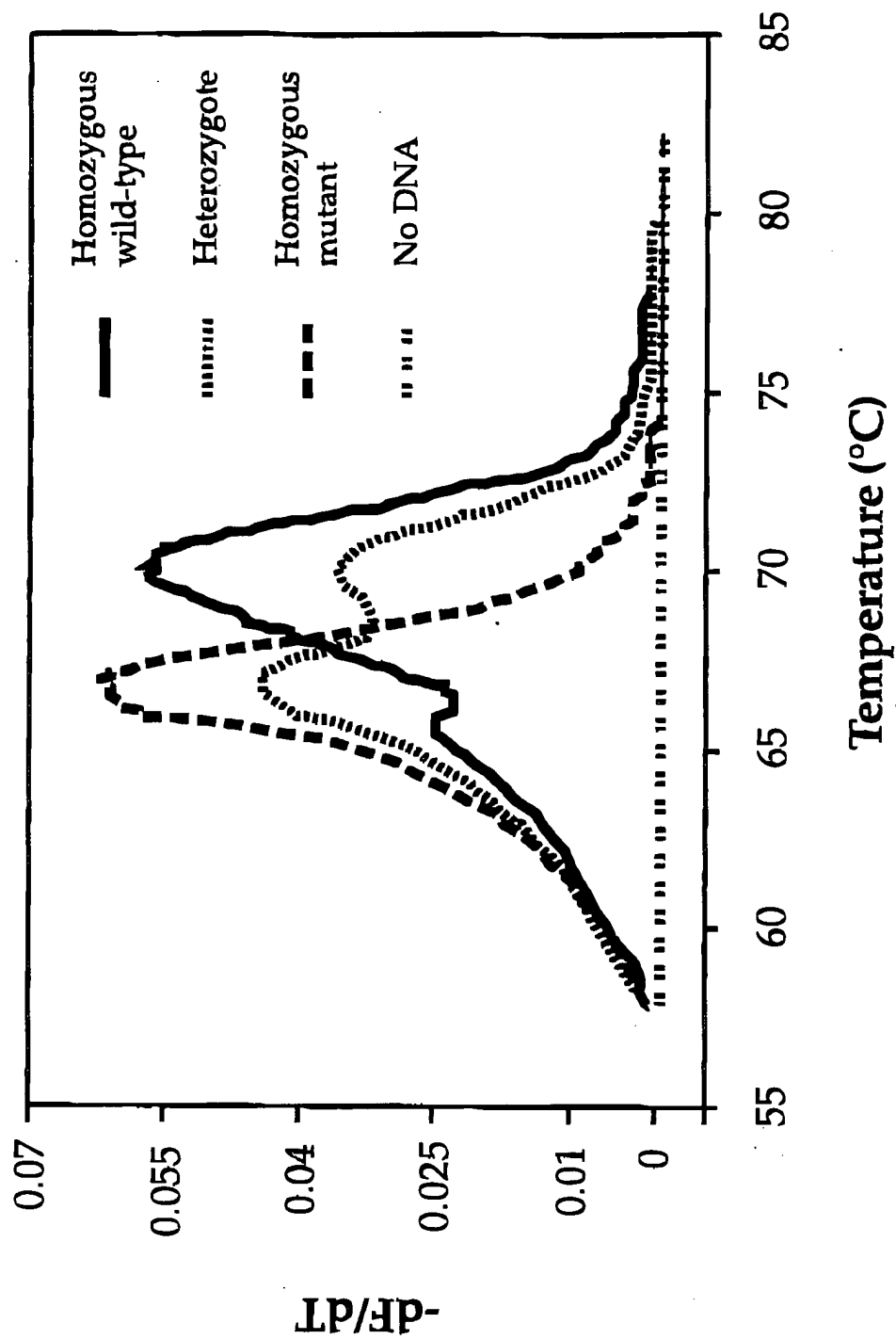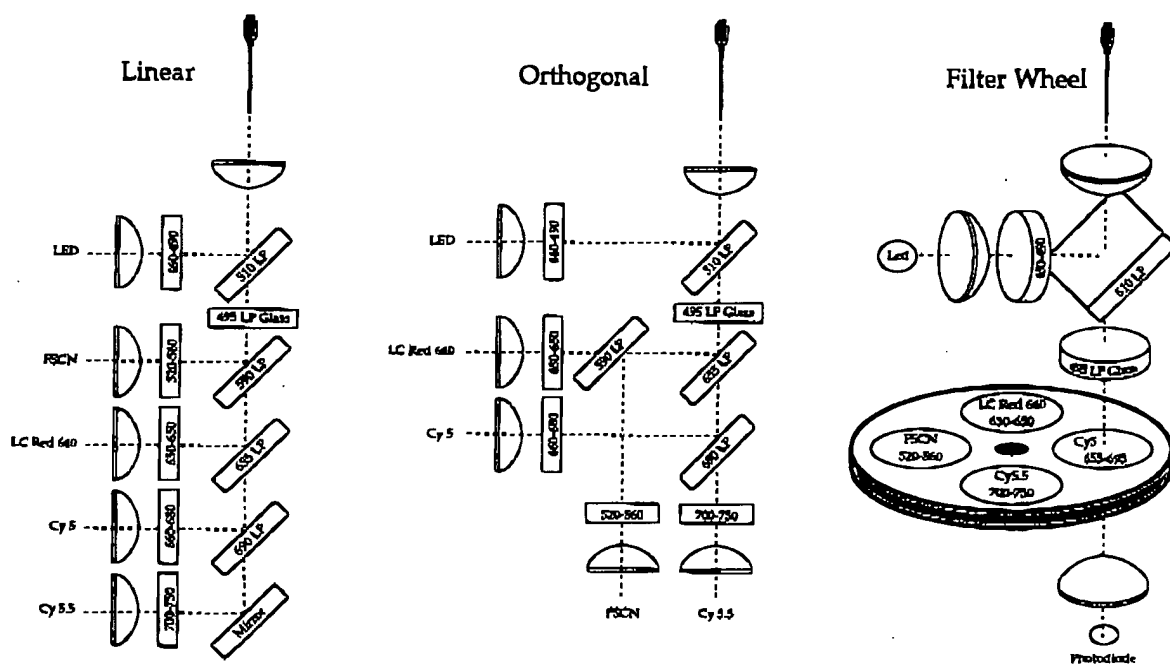
Figure 8. Four Color Light Cycler™ - Optical Design

Percent of Spectra in Band Pass

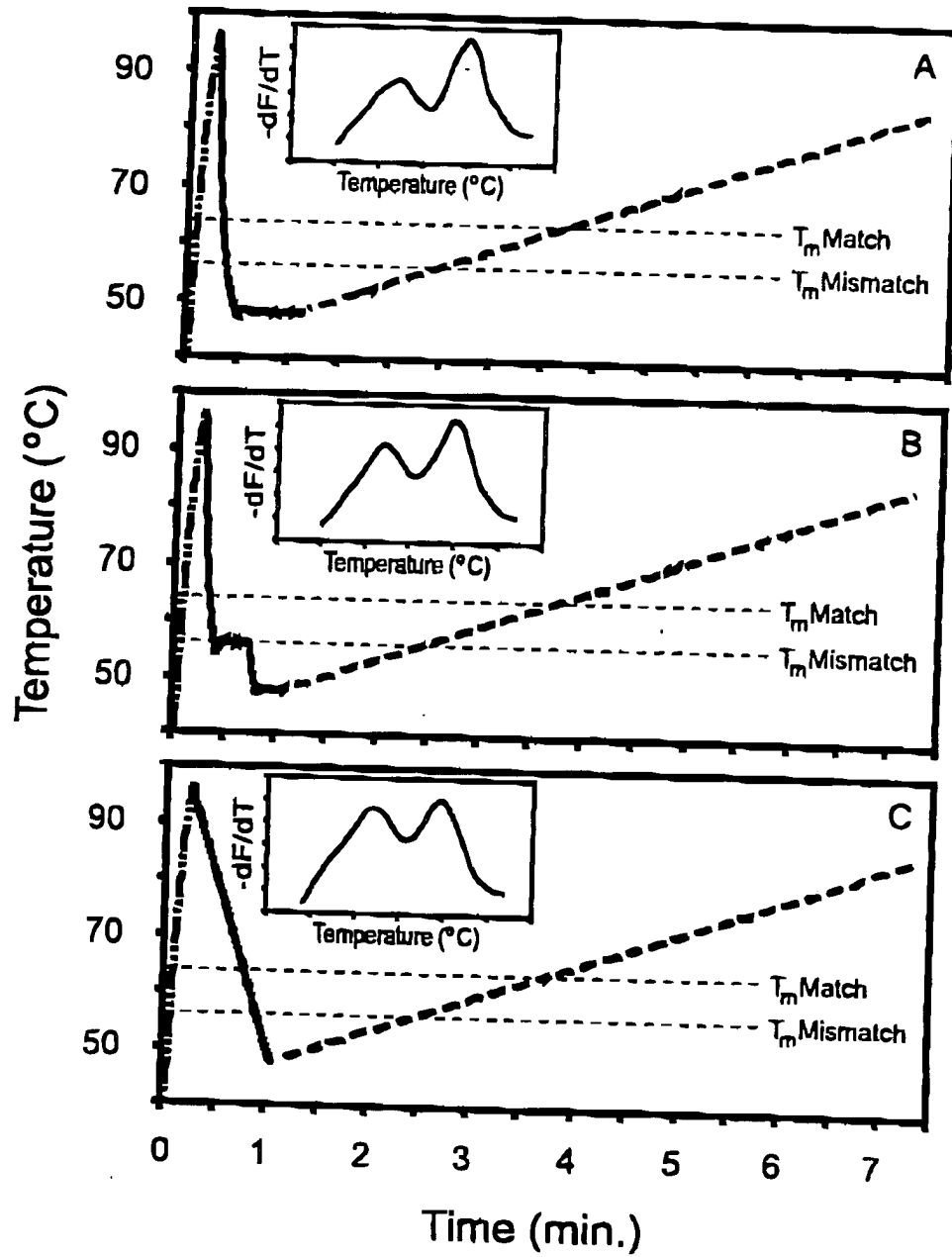|	FSCN	LC Red 640	Cy 5	Cy 5.5
LED	47	0.8	0.2	0.3
FSCN	48	<0.1	<0.1	<0.1
LC Red 640	1.5	41	7.8	0.8
Cy 5	0.7	14	43	11
Cy 5.5	0.4	7.7	16	49

Figure 10.

Figure 11.
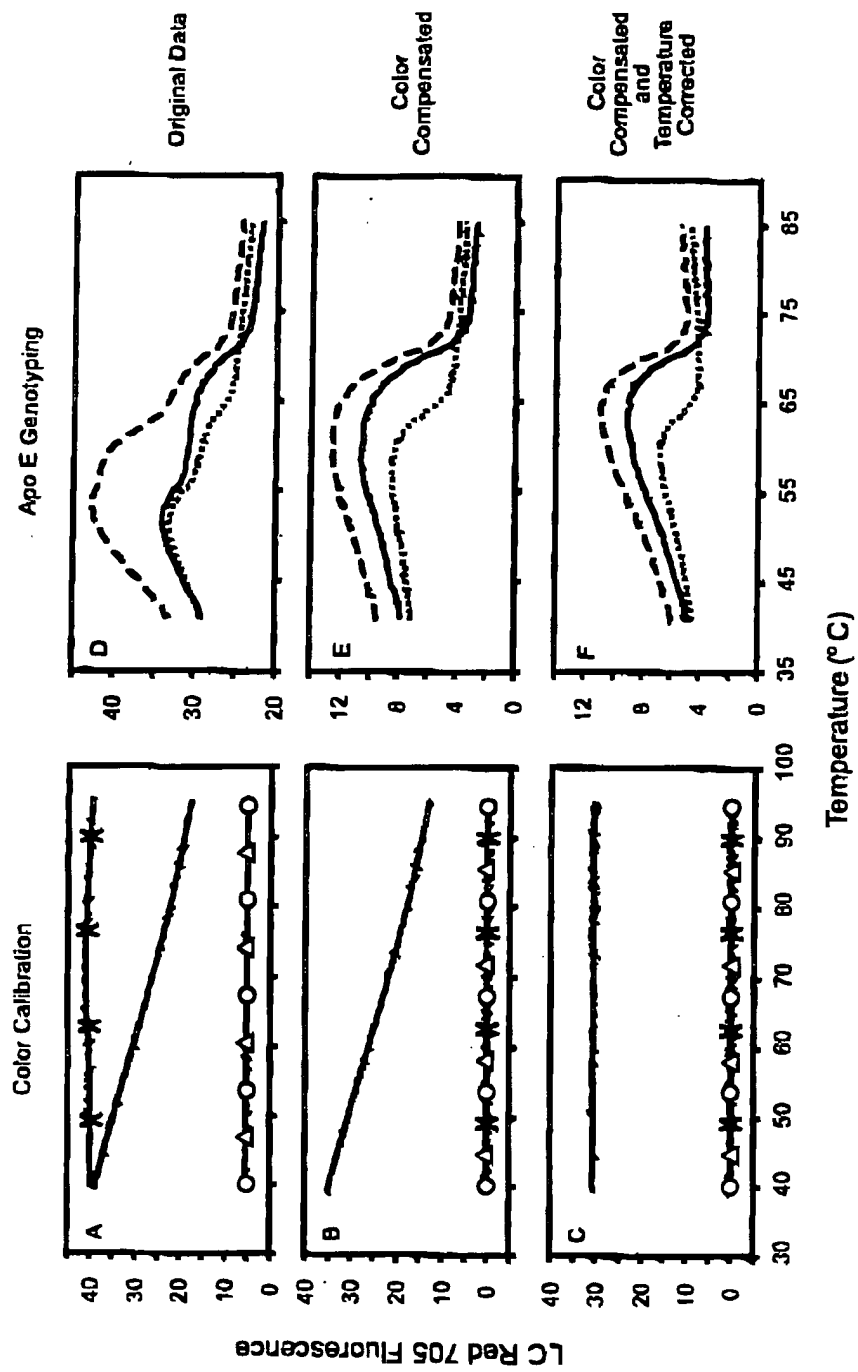
Figure 12.
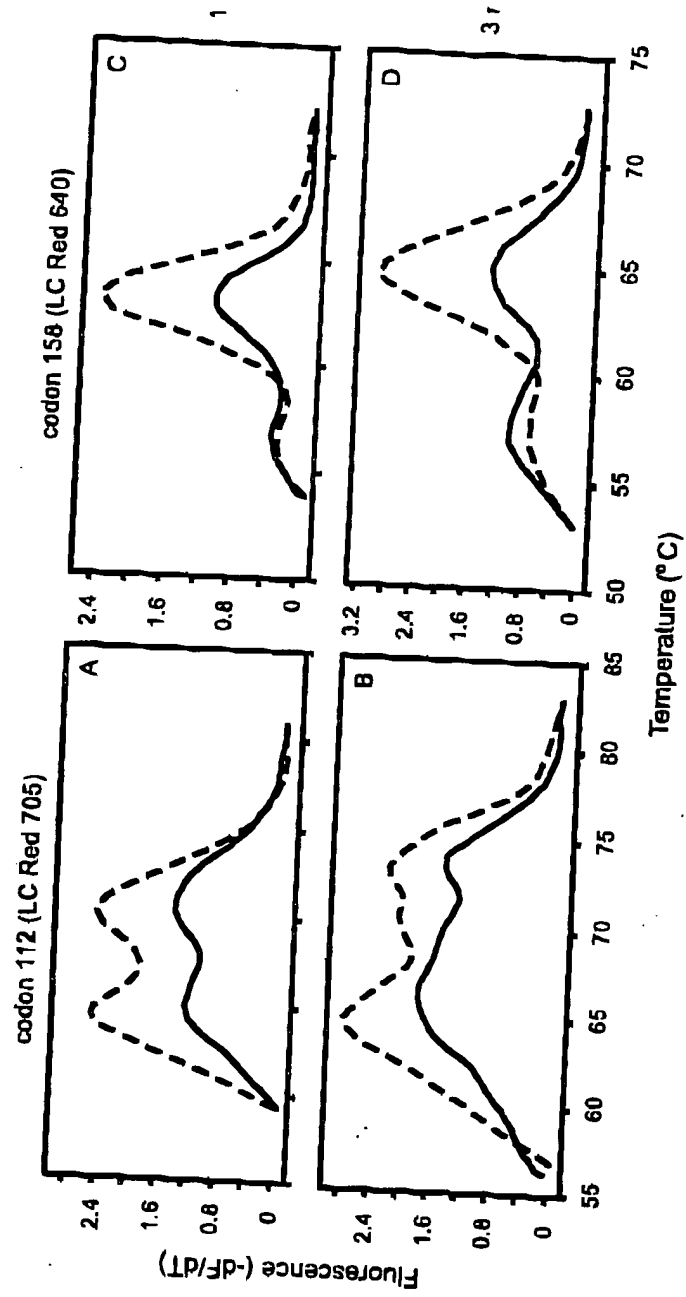
Figure 13.
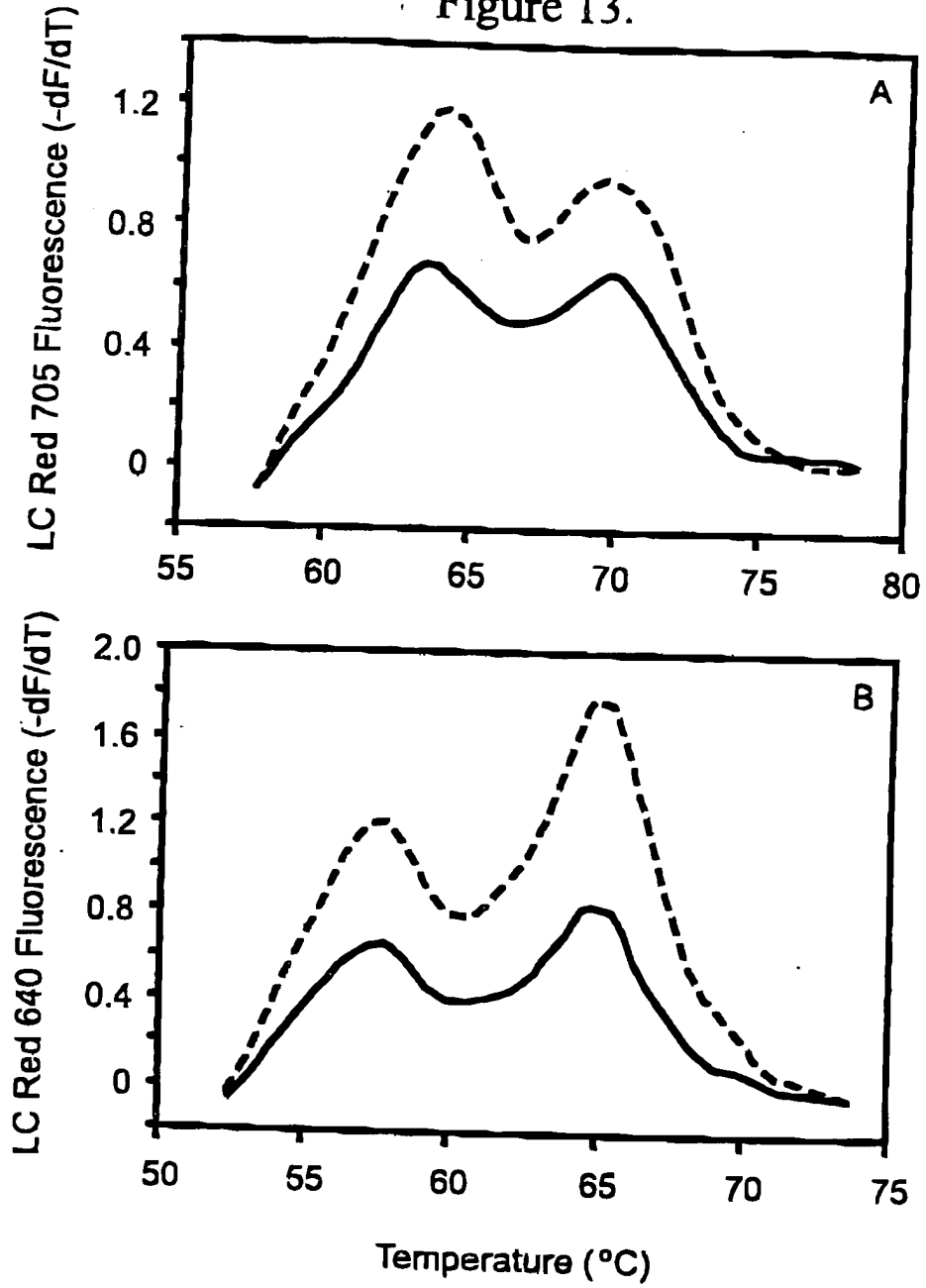
Figure 18.

Sequence Variation and Probe Design at HLA-A

```
                                                                                      GG A C   G  C AG          G
...CCGCGGGCRCCGTGGATAGRGCAGGAGRRKCCKGAGTATTGGGACCAGGAGACACGGAATATGAAGGCCCACTCA...
                                                                                       T
GGCGCCCGCGGCACCTATCTCGTCCTCCCCGGCCTCATAACC  TGGTCCTCTGTGCCTTATACTTCCGGGTGAG
                    Anchor Probe
                                      (Fluorescein)  TGCCCCTCTGTGCCTTACACTTCCGGGTGAG
                                                          Multicolored Probes
                            (LCRed640)
                            [LCRed705]
```

Matched Allele
0101
0208

Figure 19. Predicted Tms for 2 Probes at a HLA-A Variable Reigon
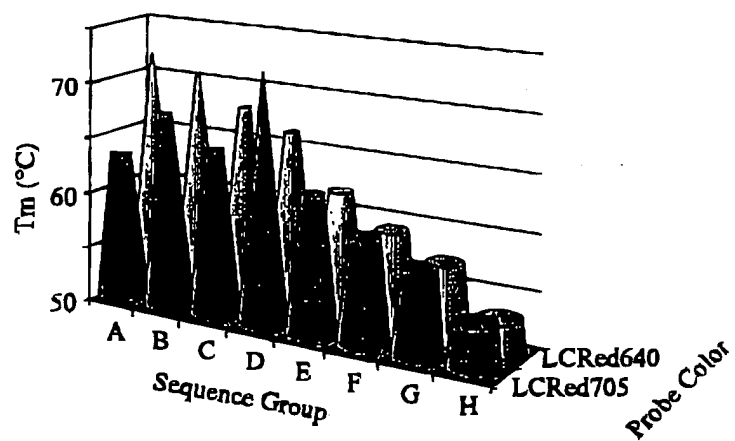
Figure 20. Sequence Variation and Probe Design at DRB1

```
...GAACAGCCAGAAGGACHTCCTGG A GCAGAGGCGGGCCGCGGT...   Matched
                              AGGCGA    C G CG                Allele
                                 C           A
        CTTGTCGGTCTTCCTGGAGGACC  CGTCTCCGCCCGGCGC           0101
            Anchor Probe          TCTGTCCGCCCGGCGC           1101
                (Fluorescein)     CGTGTTCGCCCCGGTCCA Hybrid
              (LCRed640)
                  (Cy5)               Multicolor Probes
              (LCRed705)
```

Figure 21. Predicted Tms for 3 Probes at a HLA DRB1 Variable Region
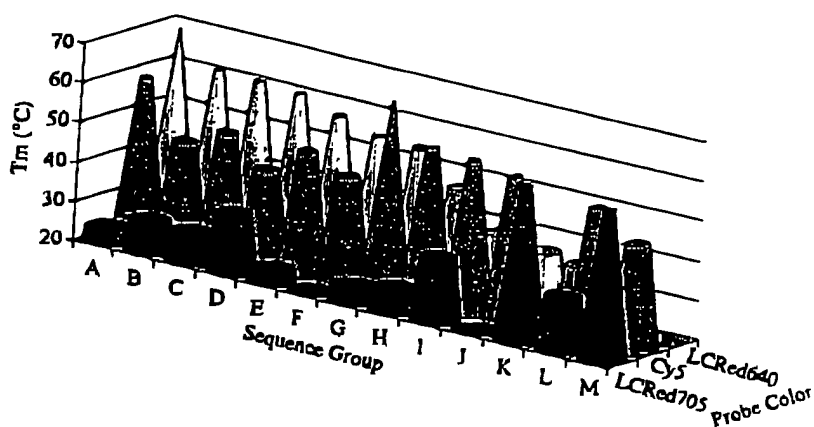
Figure 18.

Fig~~ure 28~~. Sequence Variation and Probe Design at HLA-A

```
                                                                          Matched
                                       GG A C    G  C AG        G           Allele
...CCGCGGGCRCCGTGGATAGRGCAGGAGRRKCCKGAGTATTGGGACCAGGAGACACGGAATATGAAGGCCCACTCA...      ──────
                                       T
GGCGCCCGCGGCACCTATCTCGTCCTCCCCGGCCTCATAACC  TGGTCCTCTGTGCCTTATACTTCCGGGTGAG            0101
              Anchor Probe
                                            TGCCCCTCTGTGCCTTACACTTCCGGGTGAG            0208
                                   (Fluorescein)      Multicolored Probes
                                   (LCRed640)
                                   (LCRed705)
```
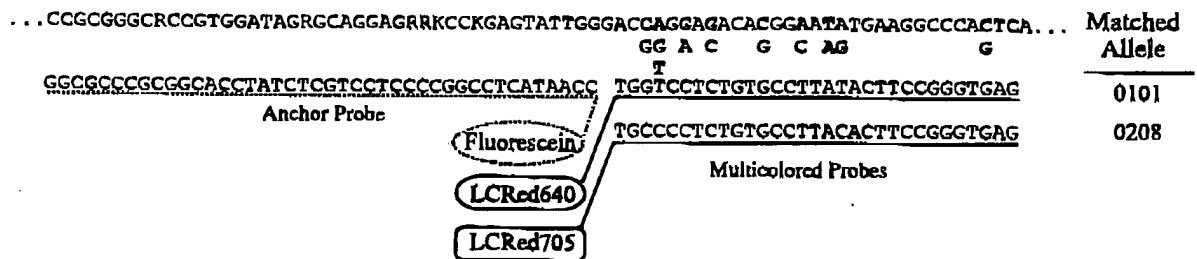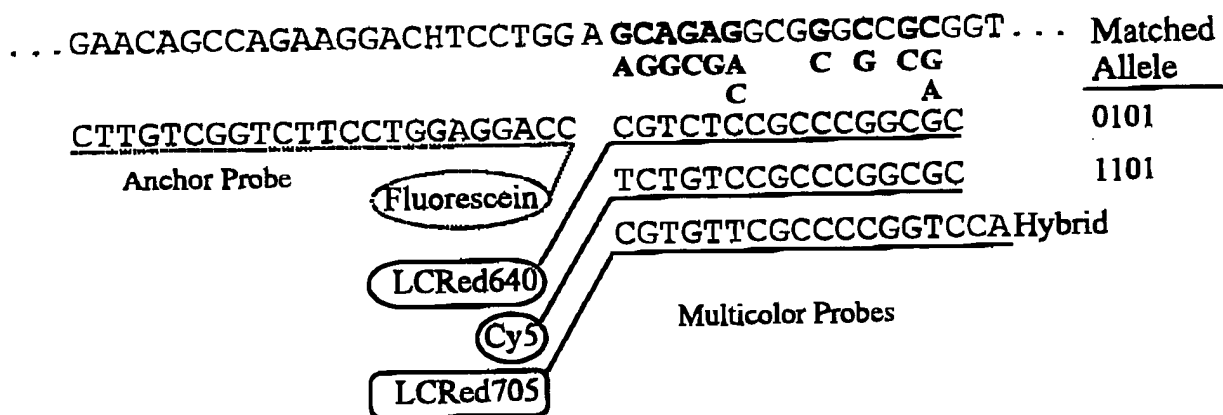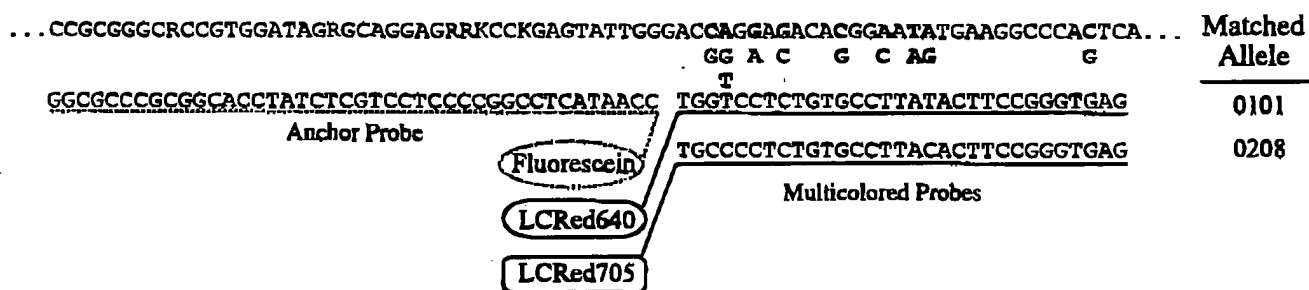
Figure 19. Predicted Tms for 2 Probes at a HLA-A Variable Reigon

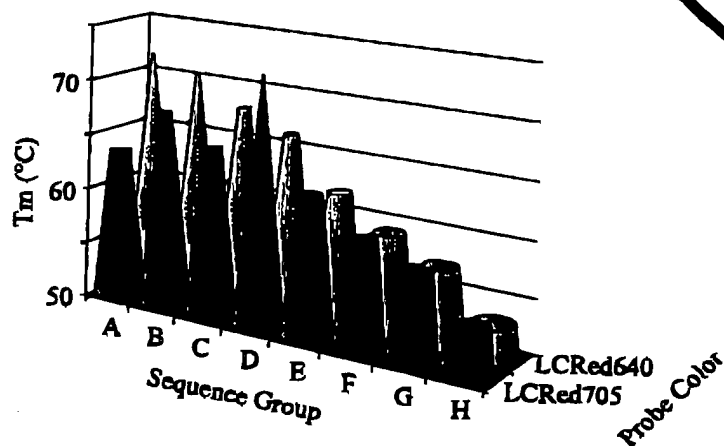| Sequence Group	HLA-A Alleles	Frequency (%)	Predicted Probe Tm	
			LCRed 640	LCRed 705
A	0101-0103 0106 3601	15.2	71.6	63.3
B	3002-3006 3101 3103 3104 3201-3204 7401-7404	6.2	70.3	67.6
C	0301 0302 0304 0305	13.4	67.8	65.0
D	0201 0202-0207 0209-0219 2408 16 additional 02XX	27.2	66.1	72.3
E	2301-2305 2402-2406 8 additional 24XX	11.4	61.6	62.5
F	2901-2903 7 additional 68XX	3.6	58.9	59.6
G	2501-2503 2601-2606 2608-2612 3301-3305	7.2	56.8	57.8
H	2502 2613 6601 6602 6801-6802 6901	~7	52.8	54.1
Total Estimated Frequency		**90.9**		

Figure 21. Predicted Tms for 3 Probes at a HLA DRB1 Variable Region

Sequence Group	DRB1 Alleles	Frequency (%)	Predicted Probe Tm		
			LCRed 640	Cy5	LCRed 705
A	0101 0404 0405 0408 0410 0472 1406	15.8	69.5	59.0	23.8
B	0801 0802 0803 0804	4.1	61.2	45.7	28.3
C	1501 1502 1503	13.6	60.9	50.4	27.8
D	0401	6.4	60.1	43.6	35.3
E	0901 1401 1404	3.8	57.1	50.6	23.5
F	1001	2.9	54.5	46.9	<20.0
G	11011 11012 1104 1305 16012 16022	7.5	54.1	68.1	24.1
H	0103 0402 1102 1103 1301 1302 1304	13.1	46.4	59.0	26.1
I	1303	1.2	57.4	58.2	36.3
J	11013 1201 1202 16011 16021	4.5	35.5	56.7	<20.0
K	0301 0302 0303 0305	10.1	34.4	<20.0	59.2
L	0403 0407	2.0	29.3	33.6	33.6
M	0701	8.2	<20.0	46.3	58.0
Total Estimated Frequency		**92.0**			